NDM-1, OXA-48 and OXA-181 carbapenemase-producing Enterobacteriaceae in Sultanate of Oman

L. Dortet[1], L. Poirel[1], F. Al Yaqoubi[2] and P. Nordmann[1]

1) *Service de Bactériologie-Virologie, INSERM U914 'Emerging Resistance to Antibiotics', Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris Sud, Le Kremlin-Bicêtre Cedex, France and* 2) *Department of Microbiology, Royal Hospital, Sultanate of Oman, Muscat, Oman*

Abstract

Twenty-two carbapenem-resistant enterobacterial isolates were recovered from patients hospitalized between October 2010 and March 2011 at the Royal Hospital of Muscat, Sultanate of Oman. Eleven NDM-1, five OXA-48 and one NDM-1 plus OXA-181 producers of diverse ST types were recovered from clinical samples. All carbapenemase genes were located on self-conjugative plasmids and were nearly always associated with other resistance determinants, including extended-spectrum β-lactamases and the ArmA methylase encoding resistance to aminoglycosides. This work highlights the dissemination of NDM-1 and OXA-48-type producers in the Middle East.

Keywords: Carbapenemase, gram-negative, NDM-1, OXA-181, OXA-48

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Corresponding author: P. Nordmann, Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France
E-mail: nordmann.patrice@bct.aphp.fr

Carbapenem-hydrolysing β-lactamases belonging to Ambler classes A, B and D have been reported worldwide among *Enterobacteriaceae*. The most clinically significant are KPC-type (Ambler class A), IMP-, VIM- and NDM-types (class B) and OXA-48 (class D), mostly identified in *Klebsiella pneumoniae* isolates as sources of nosocomial outbreaks [1].

Since its first identification from a *K. pneumoniae* isolate recovered in Turkey in 2003 [2], OXA-48 producers have been found worldwide, especially in Europe and in countries bordering the southern and eastern parts of the Mediterranean sea [1]. Several studies performed on OXA-48-producing strains revealed that the acquisition of a conserved 62.5-kilobases plasmid was at the origin of the dissemination of $bla_{\text{OXA-48}}$ in a variety of enterobacterial species, particularly in *K. pneumoniae* and *Escherichia coli* [3]. In addition, a variant of OXA-48, named OXA-181 and sharing a similar carbapenemase activity, has been identified in isolates from India or of Indian origin [4–7] with, in some cases, co-occurrence of other carbapenemases such as NDM-1 [6].

Producers of NDM-1 have been identified mainly in the UK, India and Pakistan [8], and are being increasingly reported worldwide [9]. Whereas India was considered as the main reservoir of the NDM-1-encoding gene [9], several NDM-1-producing enterobacterial isolates have been reported from the Balkan states [10] and the Middle East [11], suggesting that these areas might also be reservoirs of NDM-1 producers.

Our study was perfomed on a collection of bacterial isolates recovered at the Royal Hospital, Muscat, Sultanate of Oman, that had been elaborated for the purpose of surveillance of antimicrobial resistance mechanisms and characterization of carbapenem-resistant strains. Twenty-two multidrug-resistant *Enterobacteriaceae* (16 *K. pneumoniae*, four *E. coli*, one *Serratia marcescens* and one *Enterobacter cloacae*) isolated over a 5.5-month period (October 2010 to March 2011) were selected, all showing decreased susceptibility to carbapenems. The isolates were recovered from clinical samples obtained from patients who had been hospitalized in various units of the hospital (Table 1). PCR assays followed by sequencing were carried out for the detection of several carbapenemase genes, namely bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM} and $bla_{\text{OXA-48}}$. Ten *K. pneumoniae* and one *E. coli* were positive for $bla_{\text{NDM-1}}$ (Table 1). Two *K. pneumoniae* and three *E. coli* were positive for $bla_{\text{OXA-48}}$ (Table 1). One *K. pneumoniae* isolate co-harboured the $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-181}}$ genes (Table 1). History of travel in India could be traced for four patients from whom NDM-1-producing *K. pneumoniae* had been recovered (Table 1). For three isolates (one *S. marcescens*, one *Enterobacter cloacae* and one *K. pneumoniae*), no carbapenemase activity nor carbapenemase genes were identified, suggesting a non-carbapenemase-related resistance mechanism. Instead, there were probably combined mechanisms such as overproduction of the chromosomal

TABLE 1. Clinical features of the carbapenem resistant isolates and patient characteristics

Patient	Hospitalization unit	Underlying disease	Travel	Isolate	Date of isolation	Carbapenemase	Site of isolation	Treatment	Outcome
1	Urology	Diabetes, Fournier gangrene	No	*Klebsiella pneumoniae*	4-Nov-10	NDM-1	Wound + rectal swab	None	Improved
2	Urology	Hypospadias	No	*K. pneumoniae*	10-Dec-10	NDM-1	Urine	Meropenem + Colistin	Improved
3	General surgery	Pilonidal sinus excision	No	*Escherichia coli*	21-Dec-10	OXA-48	Wound	None	Improved
4	Acute medicine	Spinal surgery + vertebral fixation	India	*K. pneumoniae*	23-Dec-10	NDM-1	Blood + urine	Colistin + Tigecycline	Improved
5	Acute medicine	Urosepsis	India	*K. pneumoniae*	23-Dec-10	NDM-1	Blood + urine	Colistin + Tigecycline	Improved
6	Urology	Prostate carcinoma	No	*K. pneumoniae*	9-Nov-10	NDM-1	Urine	Ceftriaxone	Improved
7	Cardiothoracic surgery	Chronic cardiac diseases	No	*E. coli*	19-Dec-10	OXA-48	Blood + catheter + tracheal secretion	Meropenem + Colistin	Died
8	Acute medicine	Diabetes, parkinsonism	?	*K. pneumoniae*	1-Oct-10	NDM-1	Perineal swab + cannula site	Ciprofloxacin + Colistin	?
9	Paediatric ICU	Restrictive lung disease, diabetes	No	*Enterobacter cloacae*	11-Jan-11	None	Perineal swab	None	Improved
10	Acute medicine	Diabetes, lupus nephritis	No	*K. pneumoniae*	3-Jan-11	None	Urine + perineal swab	None	Died
11	Post coronary care unit	Diabetes, hepatitis C	No	*K. pneumoniae*	19-Jan-11	OXA-48	Wound + tracheal secretion	Piperacillin + tazobactam	Died
12	Paediatric ICU	Congenital heart disease	No	*K. pneumoniae*	2-Jan-11	None	Endotracheal secretion	Meropenem + Colistin	Improved
13	Cardiothoracic surgery	Neuromuscular disorder	India	*K. pneumoniae*	12-Feb-11	NDM-1	Wound	Surgery + Piperacillin + tazobactam	Improved
14	Paediatric ICU	Restrictive lung disease, diabetes	No	*K. pneumoniae*	15-Feb-11	OXA-48	Perineal swab	None	Improved
15	Urology	Benign prostatic hypertrophy	No	*K. pneumoniae*	28-Jan-11	NDM-1	Urine + supra-pubic catheter	Meropenem + Colistin	Improved
16	Urology	Diabetes	No	*K. pneumoniae*	8-Feb-11	NDM-1 + OXA-181	Perineal swab	None	Improved
17	Acute medicine	Diabetes, lupus nephritis	No	*K. pneumoniae*	3-Jan-11	None	Urine + perineal swab	None	Died
18	Urology	Prostatic hypertrophy	No	*K. pneumoniae*	28-Jan-11	NDM-1	Urine + supra-pubic catheter	Meropenem + Colistin	Improved
19	Paediatric unit	Liver transplant	India	*K. pneumoniae*	9-Mar-11	NDM-1	Intra-abdominal sputum	Colistin	Improved
20	Acute medicine	Diabetes, hepatitis B, liver cirrhosis	No	*E. coli*	14-Mar-11	NDM-1	Perineal swab	None	Improved
21	Acute medicine	Lupus nephritis	No	*Serratia marcescens*	24-Feb-11	None	Catheter	Tigecycline	Improved
22	General surgery	Appendectomy	No	*E. coli*	2-Feb-11	OXA-48	Rectal swab	None	Improved

ICU, intensive care unit; VAP, ventilation-assisted pneumonia; UTI, urinary tract infection.

TABLE 2. Genetic features associated with carbapenemase producers

Species/ clones	Isolates[a]	Carbapenemase	Carbapenem MICs (μg/ml)				Non β–lactam-associated resistances[b]	ST type	Plasmid carrying the carbapenemase		Associated resistance determinants	Genetic environment of bla_{NDM-1}	
			IMP	MER	ERT	DOR			Incompatibility	Size		ISAba125	ble_{MBL}
Klebsiella pneumoniae													
A	1, 2, 6	NDM-1	0.75	1.5	8	1.5	SXT, Q, Ami, TET, FT, Cm	ST147	Inc HI1B	150 kb	TEM-1, CTX-M-15, SHV-12, OXA-1, ArmA	Truncated	+
B	4, 5	NDM-1	4	16	>32	8	Q, Ami, FT, Cm	ST11	Inc L/M	150 kb	TEM-1, CTX-M-15, SHV-12, OXA-9, ArmA	Truncated	+
C	8	NDM-1	2	4	>32	2	SXT, Q, Ami, TET, FT, Cm	ST101	Untypeable	130 kb	TEM-1, CTX-M-15, OXA-1	+	+
D	13	NDM-1	3	2	4	1.5	Q, Ami	ST372	Inc FIIs	130 kb	TEM-1, CTX-M-15, SHV-28, OXA-1, OXA-9	+	+
E	15, 18	NDM-1	1.5	3	12	1.5	SXT, Q, Ami, TET, FT, Cm	ST147	Inc HI1B	200 kb	CTX-M-15, SHV-130, OXA-1, ArmA	+	+
F	19	NDM-1	4	8	24	6	SXT, Q, Ami, TET, FT	ST15	Inc HI1B	200 kb	CTX-M-15, SHV-12, OXA-1, ArmA	Truncated	+
G	11	OXA-48	0.5	0.25	0.75	0.25	SXT, Q, Ami, TET	ST754	Inc L/M OXA-48	62 kb	TEM-1, CTX-M-14, OXA-1		
H	14	OXA-48	0.5	0.38	1	0.25	None	ST753	Inc L/M OXA-48	62 kb	TEM-1		
I	16	NDM-1 OXA-181	8	16	>32	8	SXT, Q, Ami, FT, Cm	ST11	Inc HI1B	200 kb	CTX-M 15, SHV-2, OXA-1, ArmA	+	+
Escherichia coli													
A	20	NDM-1	2	8	>32	4	SXT, Q, Ami, TET	ST2527	Inc F	150 kb	TEM-1, CTX-M-15	+	+
B	3, 7	OXA-48	0.5	0.25	1	0.125	SXT, Q, Ami	ST138	Untypeable	150 kb	TEM-1, CTX-M-14		
C	22	OXA-48	0.5	0.25	1	0.25	SXT, Q, TET	ST648	Inc L/M OXA-48	62 kb	CTX-M-15		

SXT, sulfamethoxazol-trimethoprim; Q, quinolone; Ami, aminoglycosides; TET, tetracycline; FT, nitrofurantoin; Cm, chloramphenicol.
[a]Isolate numbers referred to those of patients recapitulated in Table 1.
[b]Resistance markers being co-harboured by the carbapenemase gene (bla_{NDM-1} or bla_{OXA-48}) carrying plasmid are underlined.

cephalosporinase or extended-spectrum β-lactamase associated with decreased permeability of the outer membrane.

The twenty carbapenemase-producing isolates (16 *K. pneumoniae* and four *E. coli*) were subjected to pulsed-field gel electrophoresis analysis to evaluate their clonal relationship, as previously described [11]. This analysis identified nine clones of *K. pneumoniae* and three clones of *E. coli* (Table 2). For each clone, MICs were determined by Etest (AB bioMérieux, Solna, Sweden) on Mueller–Hinton agar and the results of susceptibility testing were interpreted according to the CLSI guidelines [12]. The isolate co-producing NDM-1 and OXA-181 was fully resistant to all carbapenems, whereas all OXA-48-producing isolates harboured an apparent susceptibility to carbapenems (MICs ≤1 mg/L), making it difficult to detect with only a slight increase in carbapenem MICs (Table 2). On the other hand, NDM-1-producing isolates exhibited a variable susceptibility to carbapenems. As frequently observed for carbapenemase producers, multi-drug or pan-drug resistance was often associated. In our collection, only one isolate, an OXA-48-producing *K. pneumoniae*, did not possess a multi-drug resistance phenotype (Table 2).

Multi-locus sequence typing analysis was performed on carbapenemase-producing *K. pneumoniae* and *E. coli* isolates. Among the NDM-1-producing *K. pneumoniae*, five different ST types were identified, showing that diverse NDM-1-positive strain backgrounds circulated in the same hospital (Table 2). Two of these isolates belonged to ST147, which had been previously identified among NDM-1-producing isolates from Switzerland and Iraq, respectively [13,14]. An NDM-1-producing *K. pneumoniae* belonging to ST15 was also identified, as reported in Norway [15]. The single *K. pneumoniae* isolate co-producing NDM-1 and OXA-181 belonged to ST11, which had been previously identified in India [6]. The two OXA-48-producing *K. pneumoniae* belonged to two new STs (ST753 and ST754, respectively).

Mating-out assays was performed using clinical strains as donors and the azide-resistant *E. coli* J53 as recipient strain [7], and *E. coli* transconjugants were obtained for all donor strains (Table 2). Plasmid DNA of transconjugants was extracted by using the Kieser method [16], analysed by agarose gel electrophoresis and typed using PCR-based replicon typing [17]. In addition, the recently identified IncHI1B replicon type was sought by using recently designed primers (IncHI1B-Fw: 5′ - CAA AAC AGA GAG TAT TCA ACC C - 3′, ncHI1B-Rv: 5' - CTG ATT CTT TTC GAG ACA GGG - 3′). The bla_{NDM-1}-positive plasmids belonged to diverse incompatibility groups (Table 2). Most of those plasmids belonged to the newly identified IncHI1B group, which has been identified from NDM-1-producing *K. pneumoniae* from Morocco [18]. Most often, several resistance markers were co-harboured by the plasmid that carried the bla_{NDM-1} gene (Table 2). In all isolates except one, the bla_{OXA-48} gene was carried on a self-conjugative plasmid (62.5 kilobases) that possesses an IncL/M-type backbone on which no other antibiotic resistance gene was identified (Table 2), and corresponding to the epidemic plasmid identified worldwide [3]. The $bla_{OXA-181}$ gene was carried on an untypable and non-conjugative plasmid.

The PCR mapping showed that the bla_{NDM-1} gene was preceded by a complete or truncated copy of insertion sequence IS*Aba125* and followed by a bleomycin-resistance gene ble_{MBL}, conferring resistance to bleomycin and bleomycin-like molecules [19]. The same genetic environment has been observed for most NDM-1-positive enterobacterial isolates [20]. The bla_{OXA-48} gene was bracketed by two IS*1999* elements forming a functional composite transposon as previously described [2]. The $bla_{OXA-181}$ gene was located downstream on the insertion sequence IS*Ecp1* as described [7].

In conclusion, this study highlights the dissemination of carbapenemase producers, especially NDM-1 and OXA-48-types, in Sultanate of Oman. Emergence of those carbapenemase producers in that country might be related to the close relationship between the Arab Peninsula and India in terms of patient exchange.

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Transparency Declaration

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References

1. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 2011; 17: 1791–1798.
2. Poirel L, Héritier C, Tolun V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004; 48: 15–22.

3. Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. *Antimicrob Agents Chemother* 2012; 56: 559–562.
4. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the sentry antimicrobial surveillance program, 2006–2007. *Antimicrob Agents Chemother* 2011; 55: 1274–1278.
5. Kalpoe JS, Al Naiemi N, Poirel L, Nordmann P. Detection of an Ambler class D OXA-48-type β-lactamase in a *Klebsiella pneumoniae* strain in the Netherlands. *J Med Microbiol* 2011; 60: 677–678.
6. Poirel L, Ros A, Carricajo A *et al.* Extremely drug-resistant *Citrobacter freundii* isolate producing NDM-1 and other carbapenemases identified in a patient returning from India. *Antimicrob Agents Chemother* 2010; 55: 447–448.
7. Potron A, Nordmann P, Lafeuille E, Al Maskari Z, Al Rashdi F, Poirel L. Characterization of OXA-181, a carbapenem-hydrolyzing class D β-lactamase from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2011; 55: 4896–4899.
8. Kumarasamy KK, Toleman MA, Walsh TR *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010; 10: 597–602.
9. Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. *Trends Microbiol* 2011; 19: 588–595.
10. Livermore DM, Walsh TR, Toleman M, Woodford N. Balkan NDM-1: escape or transplant? *Lancet Infect Dis* 2011; 11: 164.
11. Poirel L, Al Maskari Z, Al Rashdi F, Bernabeu S, Nordmann P. NDM-1-producing *Klebsiella pneumoniae* isolated in the Sultanate of Oman. *J Antimicrob Chemother* 2010; 66: 304–306.
12. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. M100-s21*. Wayne, PA: CLSI, 2010.
13. Poirel L, Fortineau N, Nordmann P. International transfer of NDM-1-producing *Klebsiella pneumoniae* from Iraq to France. *Antimicrob Agents Chemother* 2011; 55: 1821–1822.
14. Poirel L, Schrenzel J, Cherkaoui A, Bernabeu S, Renzi G, Nordmann P. Molecular analysis of NDM-1-producing enterobacterial isolates from Geneva, Switzerland. *J Antimicrob Chemother* 2011; 66: 1730–1733.
15. Samuelsen O, Thilesen CM, Heggelund L, Vada AN, Kummel A, Sundsfjord A. Identification of NDM-1-producing *Enterobacteriaceae* in Norway. *J Antimicrob Chemother* 2011; 66: 670–672.
16. Kieser T. Factors affecting the isolation of ccc DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* 1984; 12: 19–36.
17. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; 63: 219–228.
18. Poirel L, Benouda A, Hays C, Nordmann P. Emergence of NDM-1-producing *Klebsiella pneumoniae* in Morocco. *J Antimicrob Chemother* 2011; 66: 2781–2783.
19. Dortet L, Nordmann P, Poirel L. Association of the emerging carbapenemase NDM-1 to bleomycin resistance protein in *Enterobacteriaceae* and *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2012; (In press).
20. Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetics features of bla_{NDM-1}-positive *Enterobacteriaceae*. *Antimicrob Agents Chemother* 2011; 55: 5403–5407.